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Chapter 2

MITOCHONDRIAL POPULATION GENETICS INFERENCES ABOUT THE PHYLOGEOGRAPHY AND SYSTEMATICS OF THE TAYRA (*EIRA BARBARA*, MUSTELIDAE, CARNIVORA)

Manuel Ruiz-García[1,*], Nicolás Lichilín-Ortíz[1], Yolanda Mejia[1], Juan Manuel Ortega[1] and Joseph Mark Shostell[2]

[1]Laboratorio de Genética de Poblaciones-Biología Evolutiva, Unidad de Genética. Departamento de Biología, Facultad de Ciencias. Pontificia Universidad Javeriana, Bogotá DC, Colombia

[2]Department of Math Science and Technology, University of Minnesota Crookston, Crookston, MN, US

[*] Correspondence: mruizgar@yahoo.es, mruiz@javeriana.edu.co.

ABSTRACT

We sequenced the mitochondrial (mt) *ND5* gene of 100 specimens of *Eira barbara* (Mustelidae, Carnivora). The samples represented six out of the seven putative morphological subspecies recognized for this Mustelidae species (*E. b. inserta*, *E. b. sinuensis*, *E. b. poliocephala*, *E. b. peruana*, *E. b. madeirensis*, and *E. b. barbara*) throughout Panama, Colombia, Venezuela, French Guiana, Brazil, Ecuador, Peru, Bolivia, Paraguay, and Argentina. The main results show that the genetic diversity levels for the overall samples and within each one of the aforementioned putative taxa were very high. The phylogenetic analyses showed that the ancestor of the Central and South-American *E. barbara* originated during the Miocene or Pliocene (6.3-4 millions of years ago, MYA). Furthermore, the ancestors of some geographical groups, (we detected at least four) originated during the Pliocene (3.7-2.5 MYA). These four groups (or lineages) were placed in the Cesar-Antioquia Departments (northern Colombia), Bolivia and northwestern Argentina, northern-central Peru, and in the trans-Andean area of Ecuador. However, during the Pleistocene, this species experienced a strong population expansion and many haplotypes expanded their geographical distributions. They became superimposed on the geographical areas of older geographical groups that originally differentiated during the Pliocene. Until new molecular studies are completed, including those with nuclear markers, we proposed the existence of only two subspecies of *E. barbara* (*E. b. inserta* in southern Central America, and *E. b. barbara* for all South America). All of the demographic analyses showed a very strong population expansion for this species in the last 400,000 YA during the Pleistocene.

Keywords: Tayra, *Eira barbara*, mitochondrial *ND5* gene, putative geographical subspecies, genetic diversity, phylogeography, population expansion during the Pleistocene

INTRODUCTION

Tayra (*Eira barbara*) is a Mustelidae (Carnivora, Mammalia) with a long, and slender body. Its length varies from 56 to 71 cm, not including a 37 to 46 cm long bushy tail. Its weight ranges from 2.7 to 7 kg with males larger than females. This species has short, dark brown to black fur that is

relatively uniform across the body, limbs, and tail, except for a yellow or orange spot on the chest. The fur on the head and neck is much paler, typically tan or greyish in color. The head has small, rounded, ears, long whiskers and black eyes with a blue-green shine. The feet have toes of unequal length with tips that form a strongly curved line when held together. The claws are short and curved, but strong, being adapted for climbing and running rather than digging.

This species occurs from southern Veracruz (Mexico) throughout Central America and across South America to northern Argentina save for the high Andes and the Caatinga and Cerrado (eastern Brazil; Emmons and Feer 1990). It is one of the most common medium-size predators throughout its range (Emmons and Feer 1990).

E. barbara is a diurnal, sometimes crepuscular species (González-Maya et al., 2015), with a solitary behavior and large home range (Sunquist et al., 1989). Emmons and Feer (1990) showed that the tayra inhabits tropical and subtropical forests, secondary rain forests, gallery forests, gardens, cloud forests, and dry scrub forests. Hall and Dalquest (1963) affirmed that it can live near human disturbed habitats. It frequently occurs in agricultural areas and along the edge of human settlements. Tayra usually inhabits areas below 1,200 m, but there are reports of it being in areas as high as 2,400 m (Eisenberg 1989, Emmons and Feer 1990) and it is common at 2,000 m (Cuarón et al., 2016). Its diet is omnivorous, including fruits, carrion, small vertebrates, insects, honey and small vertebrates such as marsupials, rodents, and iguanids among others (Cabrera and Yepes 1960, Hall and Dalquest 1963, Emmons and Feer 1990). This species is listed as Least Concern (Cuarón et al., 2016).

Cabrera (1957) and Hall (1981) recognized seven morphological subspecies, two in Central America and five in South-America: 1- *E. b. senex* (Thomas in 1900). The type locality is Hacienda Tortugas, Jalapa, Veracruz, Mexico; 2- *E. b. inserta* (Allen in 1908), with the type locality in Ulse, Matagalpa, Nicaragua; 3- *E. b. sinuensis* (Humboldt in 1812), with the type locality for the Sinu River in the Bolivar Department in northern Colombia; 4- *E. b. poliocephala* (Traill in 1821), with type locality Demerara in Guyana; 5- *E. b. madeirensis* (Lonnberg in 1913), with type

locality in Humaitá, Madeira River, Brazilian Amazon; 6- *E. b. peruana* (Nehring in 1886), with type locality in YuracYaku in the San Martin Department in Peru and 7- *E. b. barbara* (Linnaeus in 1758) with the type locality assigned by Lonnberg (1913) to Pernanbuco, Brazil. See Figure 1.

Although it is a relatively common species, only one preliminary study on its molecular population genetics and infra-specific systematics has been published (Ruiz-García et al., 2013).

Therefore, we expanded upon our initial molecular population genetics study with mitochondrial genes of the tayra with the following main aims: 1- To estimate the mitochondrial levels of genetic diversity in the overall tayra population and in some putative morphological subspecies; 2- To determine if there is a correlation between the molecular clades obtained in the phylogenetic analyses with the traditional putative morphological and geographical subspecies of tayras; 3- To estimate the possible temporal splits in the mitochondrial diversification within the evolution of the tayra; and 4- To determine if demographic evolutionary changes have characterized the natural history of the tayra.

MATERIALS AND METHODS

Samples

We sequenced 100 tayras at the mt *ND5* gene. The samples came from 11 countries and represent seven of the eight putative morphological subspecies (Table 1 & Figure 1). They are: 1- Argentina, eight individuals (putative *E. b. barbara*); 2- Bolivia, 16 specimens (putative *E. b. barbara*); 3- Brazil, nine exemplars (four putative *E. b. barbara*; five putative *E. b. madeirensis*); 4- Colombia, 12 individuals (three putative *E. b. madeirensis*; nine putative *E. b. sinuensis*); 5- Ecuador, 27 specimens (four putative *E. b. sinuensis*; 23 putative *E. b. madeirensis*); 6- French Guiana, five exemplars (putative *E. b. poliocephala*); 7- Panama, one individual (putative *E. b. inserta*); 8- Paraguay, four specimens (putative *E. b. barbara*); 9- Peru, 17 exemplars (nine putative *E. b. peruana*; eight

putative *E. b. madeirensis*); 10- Trinidad and Tobago, one individual (putative *E. b. poliocephala*). Thus, these samples represent six out of the seven putative morphological subspecies recognized for this species.

The DNA of some of the tayra individuals we analyzed was extracted from hairs obtained from animals found alive in diverse Indian communities throughout Central and South America. Another fraction of the DNA was obtained from skins, bones, and teeth of hunted individuals of *E. barbara*. We requested permission to collect biological materials from these skins, bones, and teeth that were already present in the Indian communities. In the case of the skins, we sampled 1-2 cm^2. Communities were visited only once. All sample donations were voluntary, and no financial or other incentive was offered for supplying specimens for analysis. For more information about sample permissions, see the Acknowledgment section.

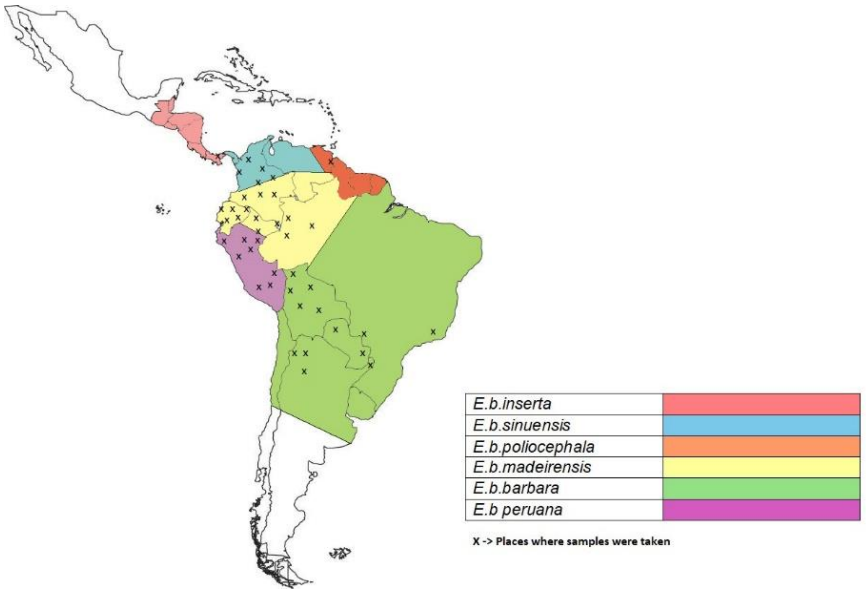


Figure 1. Map with the approximate geographical distributions of the six putative geographical tayra's subspecies (*Eira barbara*) sequenced at the mitochondrial *ND5* gene. X represent localities where samples were obtained.

Table 1. Samples of taya (*Eira barbara*), by countries, localities and putative geographic subspecies, sequenced at the mitochondrial *ND5* gene for this work

Country	Number of samples studied	Localities	Putative geographic subspecies
Panama	1	1 Chiriqui	*E. b. inserta*
Colombia	12	2 Agustín Codazzi-Cesar 1 PNN Los Katios-Chocó 1 Zaragoza-Antioquia 1 Yarumal-Antioquia 1 PNN Tama, Norte de Santander 2 El Tuparro-Vichada 1 San Martín-Meta 1 Playa Blanca-Guainia 1 Puerto Arara-Amazonas 1 Leticia-Amazonas	9 *E. b. sinuensis* 3 *E. b. madeirensis*
Trinidad & Tobago	1	1 Rio Claro	*E. b. poliocephala*
French Guiana	5	5 Camopi River	*E. b. poliocephala*
Ecuador	27	3 Yarinacocha-Pastaza 2 Sucua-Morona Santiago 2 La Perla-Santo Domingo de Tsáchilas 2 Miashi-Zamora 2 Pillaro-Tungurahua 2 Canelos-Pastaza 2 Sarayaku-Pastaza 1 Coca-Napo 1 Misahuallí-Napo 1 La Bonita-Napo 1 Macuma-Morona Santiago 1 Loreto-Napo 1 Hushafindi-Napo 1 Pichincha 1 Miazal-Morona Santiago	4 *E. b. sinuensis* 22 *E. b. madeirensis*

Country	Number of samples studied	Localities	Putative geographic subspecies
Ecuador		1 Yangana-Loja 1 Cononaco-Pastaza 1 Tinigua-Napo 1 Nuevo Rocafuerte-Napo	
Peru	17	2 Iquitos-Loreto 1 Caballococha-Loreto 1 Nauta-Loreto 1 Luceropata-Loreto 1 Puerto Venus-Loreto 1 Lamas-San Martin 1 Moyobamba-San Martin 1 Rioja-San Martin 1 Nuevo Cajamarca-San Martin 1 Bagua Grande-Amazonas 1 Oxamarca-Cajamarca 1 Puerto Bermudez-Pasco 1 Bolognesi-Ucayali 1 Seshea-Ucayali 1 Manu-Madre de Dios 1 Marcapata-Cusco	8 *E. b. madeirensis* 9 *E. b. peruana*
Bolivia	16	3 Ballivian-Beni 1 Piso Firme-Beni 1 Nicolas Suarez-Pando 1 Sena-Pando 1 Franz Tamayo-La Paz 1 Coripata-La Paz 1 Cajuata-La Paz 1 Totora-Cochabamba 1 Pojo-Cochabamba 1 Vila vila-Cochabamba 1 Julpe River-Cochabamba 1 El Cerro-Santa Cruz 1 Puerto Pailas-Santa Cruz 1 San Jose de Chuiquitos-Santa Cruz	*E. b. barbara*

Table 1. (Continued)

Country	Number of samples studied	Localities	Putative geographic subspecies
Brasil	9	3 Foz de Iguazu-Parana 3 Novo Airao-Negro River-Amazonas 1 Moora-Negro River-Amazonas 1 Paumari-Yavari River-Amazonas 1 Tres Rios-Rio de Janeiro	4 *E. b. barbara* 5 *E. b. madeirensis*
Paraguay	4	2 Los Cedrales 2 Loma Grande	*E. b. barbara*
Argentina	8	2 Salta 1 Abra Pampa-Jujuy 1 Humahuaca-Jujuy 1 La Cocha-Tucuman 1 Burruyacu-Tucuman 1 El Dorado-Misiones 1 San Javier-Misiones	*E. b. barbara*

Molecular Analyses

The DNA from skins and bones was extracted using the phenol-chloroform procedure (Sambrook et al., 1989), whereas DNA samples from hairs and teeth were extracted with 10% Chelex resin (Walsh et al., 1991). Primers and PCR conditions for the *ND5* gene (265 bp) were brought to a volume of 25 µl with 13.5 µl of Mili-Q H_2O, 3 µl of $MgCl_2$ 1 mM, 1 µl of dNTPs 0.2 mM, 1 µl of each primer (0.1 µM), 2.5 µl of buffer 10X, and one unity of Taq Polymerase with 50-100 ng of DNA. We used the primers L12673 and H12977 (5'-GGTGCAACTC CAAATAAAAGTA -3' and 5´- AGAATTCTATGATGGATCATGT 3'; Waits et al., 1999). The PCR temperatures were 95° for 5 minutes followed by 10 cycles of 1 minute at 95°C, 1 minute at 64°C and 1.5 minute at 72°C, 25 cycles of 1 minute at 95°C, 1 minute at 60°C and 1.5 minute at 72°C

and one final extension of 15 minutes at 72°C. All amplifications, including positive and negative controls, were checked in 2% agarose gels. Those samples that amplified were purified using membrane-binding spin columns (Qiagen). The PCR products were sequenced in both directions using the Big Dye™ kit in an ABI 377A automated DNA sequencer. A consensus of the forward and reverse sequences was determined using the Sequencher program.

It is possible that some of the sequences represent numts (mitochondrial DNA fragments inserted into the nuclear genome) rather than true mtDNA (Chung and Steiper, 2008). However, we note that all amino acid translations of the obtained sequences showed the presence of initial start and terminal stop codons and the absence of premature stop codons. Protein translation was also checked to evaluate the possible presence of numts. Nevertheless, the mutations we observed were synonymous changes, thus suggesting that there were no numts in the sequences.

Data Analyses

Genetic Diversity

The statistics used to determine the genetic diversity in the overall tayra sample and within the five South American putative tayra subspecies were as follows: the haplotypic diversity (H_d), the nucleotide diversity (π), the average number of nucleotide differences (K), and the θ statistic by sequence. These statistics were obtained using the DNAsp 5.1 software (Librado and Rozas, 2009).

Phylogenetics Analyses

The sequence alignments were carried out manually as well as with the DNA Alignment program (Fluxus Technology Ltd.). MrModeltest v2.3 software (Nylander, 2004) and Mega 6.05 software (Tamura et al., 2013) were applied to determine the best evolutionary mutation model. The Akaike and Bayesian information criteria (AIC and BIC; Akaike,

1974; Schwarz, 1978) were used to determine the best evolutionary nucleotide model in the overall sequence set of *E. barbara*.

Phylogenetic trees were constructed by using two procedures: Maximum Likelihood (MLT) and Bayesian analysis (BI). The ML trees were obtained using the RAxML v.7.2.6 software (Stamatakis, 2006). To select the best fitting model, 50 independent iterations were run using three data partitions (codon 1, codon 2, and codon 3). Additionally, 50 iterations were run using two data partitions (codons 1+2 combined, and codon 3). For each sequence data set, the GTR + G model (General Time Reversible + gamma distributed rate variation among sites; Tavaré, 1986) was used to search for the ML tree and topologic support was estimated with 500 bootstrap replicates using GTR.

A BI tree was completed with the BEAST v. 1.8.1 program (Drummond et al., 2012). Four independent iterations were run using three data partitions (codon 1, codon 2, and codon 3) with six MCMC chains sampled every 10,000 generations for 30 million generations after a burn-in period of 3 million generations. We checked for convergence using Tracer v1.6 (Rambaut et al., 2013). We plotted the likelihood versus generation and estimated the effective sample size (ESS > 200) of all parameters across the four independent analyses to determine convergence and optimal results. The results from different runs were combined using LogCombiner v1.8.0 and TreeAnnotator v1.8.0 software (Rambaut and Drummond, 2013). A Yule speciation model and a relaxed molecular clock with an uncorrelated log-normal rate of distribution (Drummond et al., 2006) were used. Posterior probability values provide an assessment of the degree of support of each node on the tree. The tree was visualized in FigTree v. 1.4 software (Rambaut, 2012). This BI tree was used to estimate the time to most recent common ancestor (TMRCA) for the different nodes. We used a prior of 24.0 $\pm$ 1 MYA (95 % confidence interval: 26.24-22.36 MYA) for the split between the ancestors of *Eira* and one Procyonidae, as *Potos flavus*. This prior followed the results of Koepfli et al., (2008).

Following Pennington and Dick (2010), the previous BI temporal estimates belong to one of two different approaches for inferring

divergence times. The first approach is based on fossil-calibrated DNA phylogenies. The second approach is named "borrowed molecular clocks" and uses direct nucleotide substitution rates inferred from other taxa. For this second approach, we used a median joining network (MJN) with the help of Network 4.6.10 software from Fluxus Technology Ltd (Bandelt et al., 1999). The ρ statistic (Morral et al., 1994) was estimated and transformed into years of divergence among the haplotypes studied. To determine the temporal splits, it is necessary to estimate a mutation rate at the mt *ND5* gene. We used a nucleotide divergence of 1.22 % per each million years (Culver et al., 2000), which yielded one mutation each 309,310 years. This estimate was obtained for Felidae. In this work, we assumed that this mutation rate could be similar in Mustelidae. The networks are more appropriate for intraspecific phylogenies than tree algorithms because they explicitly allow for the co-existence of ancestral and descendant haplotypes, whereas trees treat all sequences as terminal taxa (Posada and Crandall, 2001).

Heterogeneity Analyses

Several procedures were carried out to estimate the genetic heterogeneity among the diverse putative tayra subspecies analyzed. To determine the overall genetic heterogeneity in *E. barbara*, we used the statistics G_{ST}, γ_{ST}, N_{ST} and F_{ST} (Nei, 1973; Hudson et al., 1992). Additionally, we relied on the H_{ST}, K_{ST}, K_{ST}*, Z, Z*, and Snn tests (Hudson, 2000), and the chi-square test on the haplotypic frequencies with permutation tests using 10,000 replicates to measure genetic heterogeneity. Also, we estimated the genetic heterogeneity by subspecies pairs within *E. barbara*. For this task, we used three procedures: 1- Exact tests with Markov chains, 10,000 dememorizations parameters, 20 batches, and 5,000 iterations per batch; 2- Indirect gene flow estimates (Nm) from the F_{ST} statistic with a n-dimensional island model (Slatkin, 1985; Ruiz-García, 1993, 1994, 1997, 1999; Ruiz-García and Álvarez, 2000); and 3- Kimura 2P genetic distances (Kimura, 1980). These genetic heterogeneity statistics were completed with DNAsp 5.1 (Librado and Rozas, 2009) and Arlequin 3.5.1.2 (Excoffier and Lischer, 2010).

Demographic Changes

We relied on three procedures to detect possible historical population changes in the tayra: 1- We used the Strobeck's S statistic (Strobeck, 1987), Fu and Li D* and F* tests (Fu and Li, 1993), the Fu F_S statistic (Fu, 1997), the Tajima D test (Tajima, 1989) and the R_2 statistic (Ramos-Onsins and Rozas, 2002). A 95% confidence interval and probabilities were obtained with 10,000 coalescence permutations. 2- The mismatch distribution (pairwise sequence differences) was obtained following the method of Rogers and Harpending (1992) and Rogers et al., (1996). We used the raggedness *rg* statistic to determine the similarity between the observed and the theoretical curves. 3- A Bayesian skyline plot (BSP) was obtained by means of the BEAST v. 1.8.1 and Tracer v1.6 software. The Coalescent-Bayesian skyline option in the tree priors was selected with four steps and a piecewise-constant skyline model with 30,000,000 generations (the first 3 million discarded as burn-in), kappa with log Normal [1, 1.25] and Skyline population size with uniform [0, infinite; initial value 80]. In the Tracer v1.6, the marginal densities of temporal splits were analyzed and the Bayesian Skyline reconstruction option was selected for the trees log file. A stepwise (constant) Bayesian skyline variant was selected with the maximum time as the upper 95 % high posterior density (HPD) and the trace of the root height as the treeModel.rootHeight. To determine the time range for possible demographic changes for *E. barbara*, we consider that the evolution of this taxon occurred during the last 4 MY.

RESULTS

Genetic Diversity and Phylogenetic Inferences

The BIC showed that the best nucleotide substitution model was T92 + G (7,649.51). In contrast, the AIC detected GTR + G + I (5,881.29) as the best model.

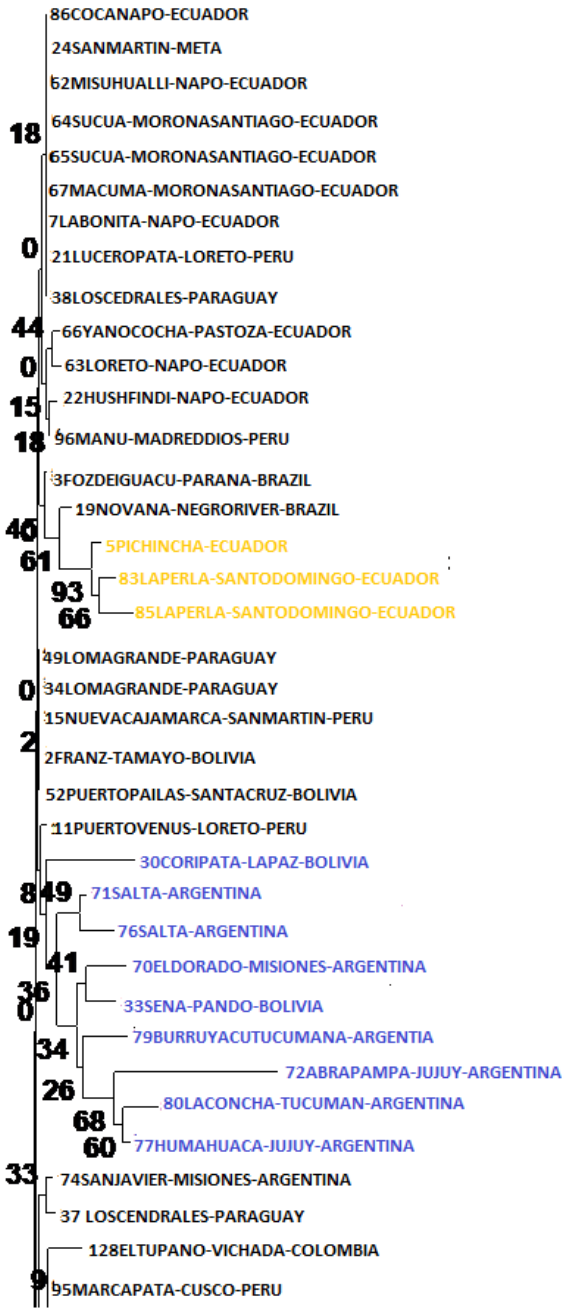
The genetic diversity levels in the overall studied sample of tayra were very high. For the 100 individuals analyzed, we found 70 different haplotypes with $H_d = 0.983 \pm 0.006$, $\pi = 0.0422 \pm 0.0048$ and $k = 11.175 \pm 5.117$. The genetic diversity for four out of five South American putative morphological subspecies were very similar, all of them with very high genetic diversity levels (H_d = 0.991-0.960 and π = 0.0562-0.0308). The genetic diversity of *E. b. poliocephala* was somewhat lower (H_d = 0.600 and π = 0.0176), although the sample size for this putative subspecies was the lowest (Table 2).

The MLT and BI can be seen in Figures 2 and 3. Both phylogenetic trees showed that the first diverging branch represented the animal sampled in northcentral Panama (putatively, *E. b. inserta*) (MLT: low bootstrap 28%; BI: p = 1). All of the South American specimens we analyzed were placed in the remaining cluster. However, although putatively animals from five different subspecies were included, very few significant clades were observed, and only partially related to the morphological subspecies. The first diverging cluster in the South American animals was one composed by three animals from northern Colombia (Cesar and Antioquia Departments; 50 % and p = 0.97, respectively), which corresponded with the putative *E. b. sinuensis.* Nevertheless, a Bolivian exemplar and many other specimens "a priori" classified as *E. b. sinuensis* by their geographical origins that did not belong to this cluster, were present in the BI, within this clade. Henceforth, there was only a partial correspondence between this clade and *E. b. sinuensis*. There were other interesting clades in both phylogenetic trees. 1- One was composed of three individuals from the Pacific area of trans-Andean Ecuador (61 % and p = 1, respectively), which also partially corresponded with *E. b. sinuensis*; 2- Another cluster was composed of individuals from different areas of Bolivia and mainly by individuals from northwestern Argentina (Salta, Jujuy and Tucuman provinces) in MLT (41 %). In the BI, this group was only composed of five individuals from northwestern Argentina (p = 0.96). This cluster was partially correlated with *E. b. barbara*. In the BI there was another cluster with several Bolivian and Argentinian specimens (p = 0.84).

It was separated from the first Argentinian cluster we aforementioned. However, as we commented for *E. b. sinuensis*, this relationship was incomplete because other individuals "a priori" classified as *E. b. barbara* were dispersed by other clusters; 3- Another cluster of certain relevance was detected in the MLT and BI. It was composed of individuals of central Peru and one individual from the northern Peruvian Amazon (80 % and p = 0.72, respectively). This cluster also partially supported the existence of the morphological subspecies *E. b. peruana*; 4- Small clusters of animals from the Ecuadorian and Colombian Amazon were present. One of them contained two animals from the Ecuadorian and Colombian Amazon (62 % and p = 1, respectively) and other three from the Ecuadorian Amazon (73 % and p = 1; 89 % and p = 1; 28 % and p = 0.8, respectively). These very locally restricted clusters were inside the putative morphological subspecies, *E. b. madereinsis*. Many other individuals of *E. b. madereinsis* were distributed in clusters with other individuals "a priori" considered different morphological subspecies.

Table 2. Genetic diversity in the overall sample of *Eira barbara* and in the five putative South American morphological subspecies at the mt *ND5* gene represented by the number of haplotypes (NH), the haplotype diversity (H_d), the nucleotide diversity (π), and the average number of nucleotide differences (K)

Eira barbara taxa	NH	H_d	π	K
Overall Sample	70	0.983 ± 0.006	0.0422 ± 0.0048	11.175 ± 5.117
E. b. sinuensis	12	0.987 ± 0.065	0.0562 ± 0.0311	14.884 ± 8.344
E. b.poliocephala	14	0.600 ± 0.147	0.0176 ± 0.0093	4.667 ± 2.556
E. b. peruana	13	0.989 ± 0.063	0.0517 ± 0.0299	13.703 ± 7.324
E. b. madeirensis	26	0.960 ± 0.009	0.0308 ± 0.0071	8.162 ± 3.233
E. b. barbara	25	0.991 ± 0.012	0.0412 ± 0.0092	10.928 ± 4.111



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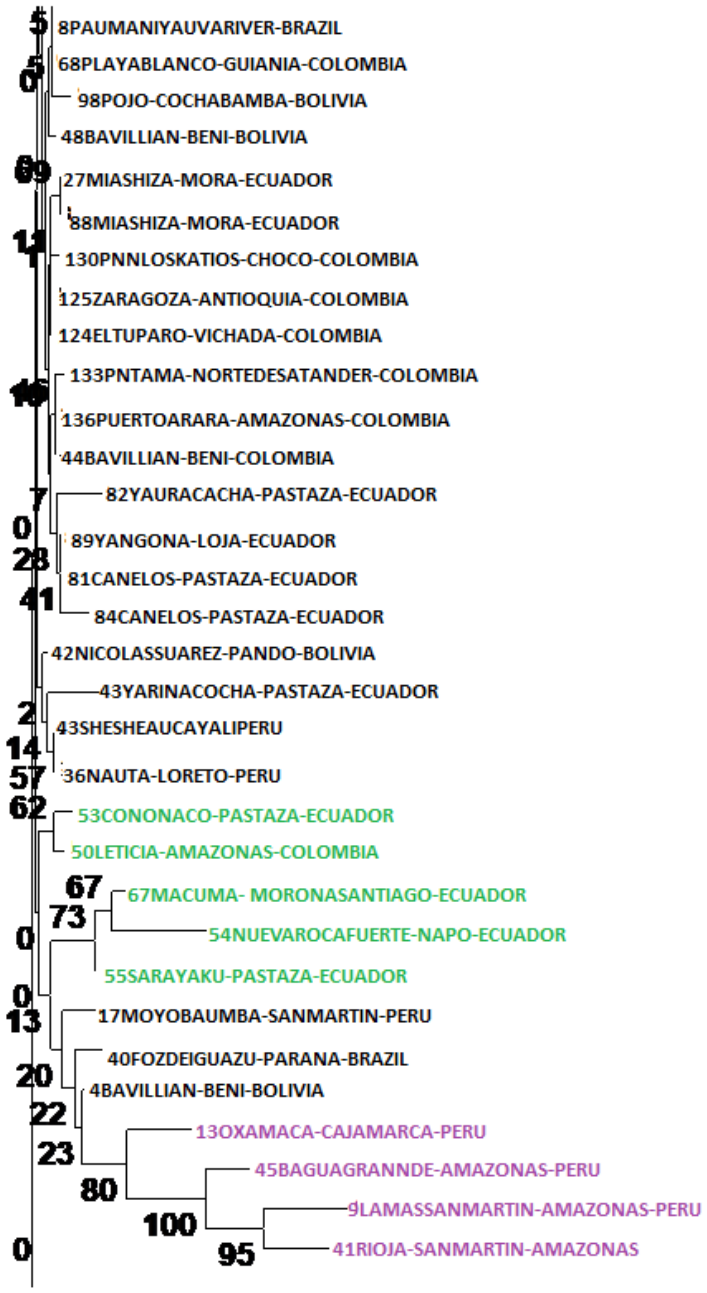
8PAUMANIYAUVARIVER-BRAZIL
68PLAYABLANCO-GUIANIA-COLOMBIA
98POJO-COCHABAMBA-BOLIVIA
48BAVILLIAN-BENI-BOLIVIA
27MIASHIZA-MORA-ECUADOR
88MIASHIZA-MORA-ECUADOR
130PNNLOSKATIOS-CHOCO-COLOMBIA
125ZARAGOZA-ANTIOQUIA-COLOMBIA
124ELTUPARO-VICHADA-COLOMBIA
133PNTAMA-NORTEDESATANDER-COLOMBIA
136PUERTOARARA-AMAZONAS-COLOMBIA
44BAVILLIAN-BENI-COLOMBIA
82YAURACACHA-PASTAZA-ECUADOR
89YANGONA-LOJA-ECUADOR
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43YARINACOCHA-PASTAZA-ECUADOR
43SHESHEAUCAYALIPERU
36NAUTA-LORETO-PERU
53CONONACO-PASTAZA-ECUADOR
50LETICIA-AMAZONAS-COLOMBIA
67MACUMA- MORONASANTIAGO-ECUADOR
54NUEVAROCAFUERTE-NAPO-ECUADOR
55SARAYAKU-PASTAZA-ECUADOR
17MOYOBAUMBA-SANMARTIN-PERU
40FOZDEIGUAZU-PARANA-BRAZIL
4BAVILLIAN-BENI-BOLIVIA
13OXAMACA-CAJAMARCA-PERU
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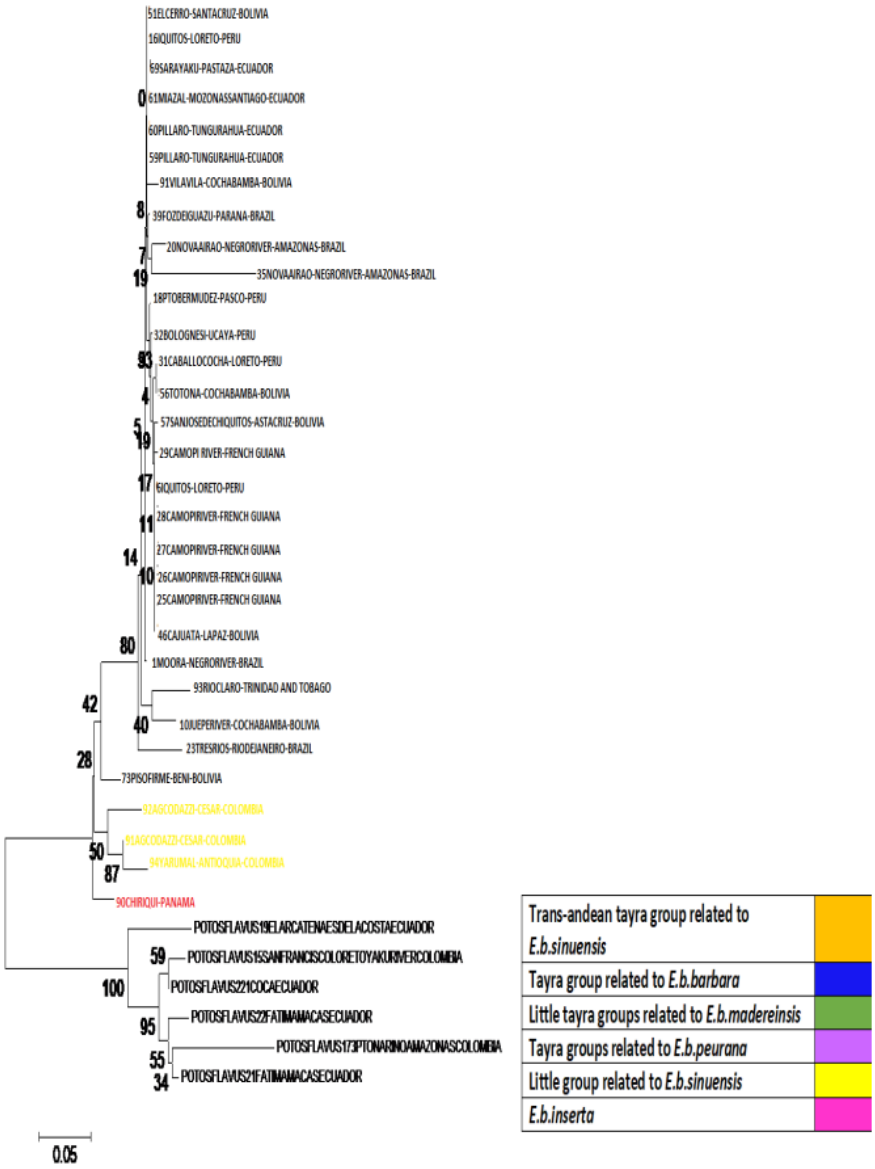
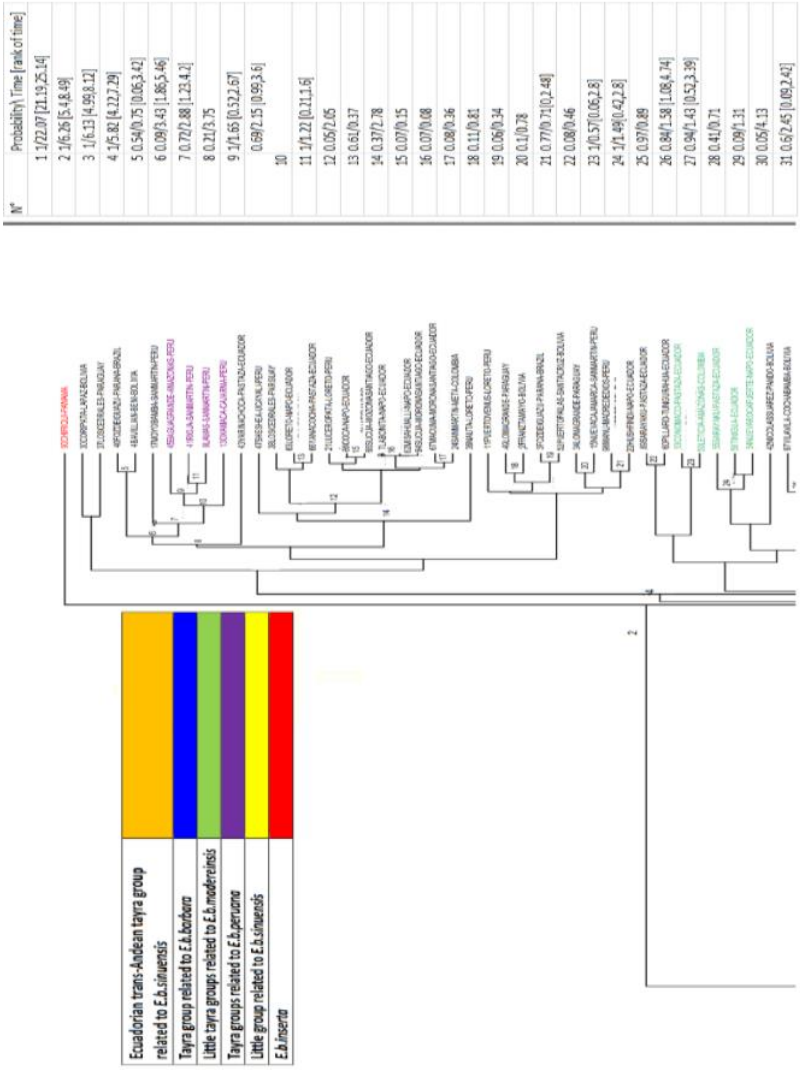
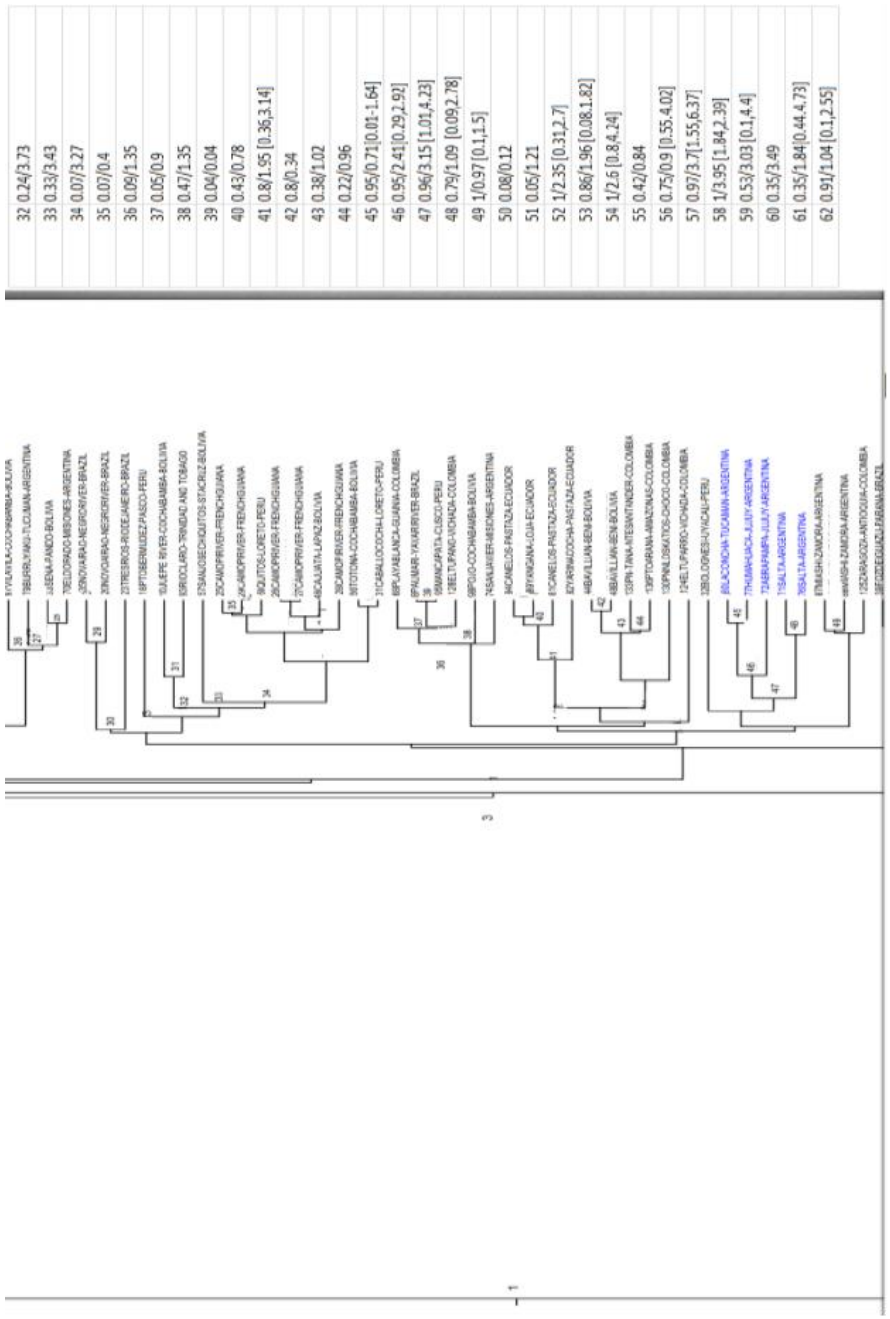


Figure 2. Maximum likelihood tree with the 100 specimens of tayra (*Eira barbara*) sequenced at the mitochondrial *ND5* gene. The number in the nodes are the bootstrap percentages. The procyonidae, *Potos flavus*, was employed as outgroup. In different

colors, some relevant clusters which showed a limited correspondence with some putative morphological geographic subspecies of *E. barbara*.

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33 0.33/3.43
34 0.07/3.27
35 0.07/0.4
36 0.09/1.35
37 0.05/0.9
38 0.47/1.35
39 0.04/0.04
40 0.43/0.78
41 0.8/1.95 [0.36,3.14]
42 0.8/0.34
43 0.38/1.02
44 0.22/0.96
45 0.95/0.71[0.01-1.64]
46 0.95/2.41[0.29,2.92]
47 0.96/3.15 [1.01,4.23]
48 0.79/1.09 [0.09,2.78]
49 1/0.97 [0.1,1.5]
50 0.08/0.12
51 0.05/1.21
52 1/2.35 [0.31,2.7]
53 0.86/1.96 [0.08,1.82]
54 1/2.6 [0.8,4.24]
55 0.42/0.84
56 0.75/0.9 [0.55,4.02]
57 0.97/3.7[1.55,6.37]
58 1/3.95 [1.84,2.39]
59 0.53/3.03 [0.1,4.4]
60 0.35/3.49
61 0.35/1.84[0.44,4.73]
62 0.91/1.04 [0.1,2.55]

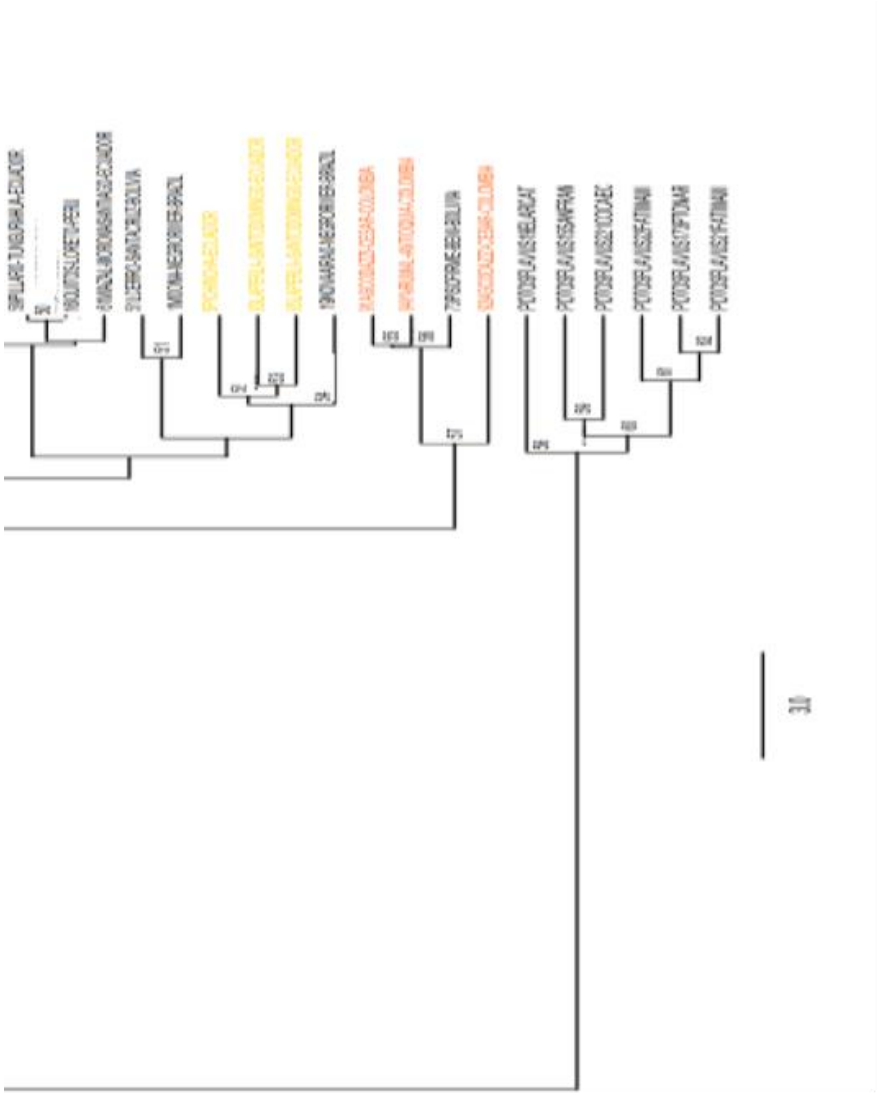

Figure 3. Bayesian tree with the 100 specimens of tayra (*Eira barbara*) sequenced at the mitochondrial *ND5* gene. The three numbers in the nodes are the posteriori probabilities, estimated temporal splits in the nodes in millions of years, and the 95% high posterior density of these temporal splits. The procyonidae, *Potos flavus*, was employed as outgroup. In different colors, some relevant clusters which showed a limited correspondence with some putative morphological geographic subspecies of *E. barbara*.

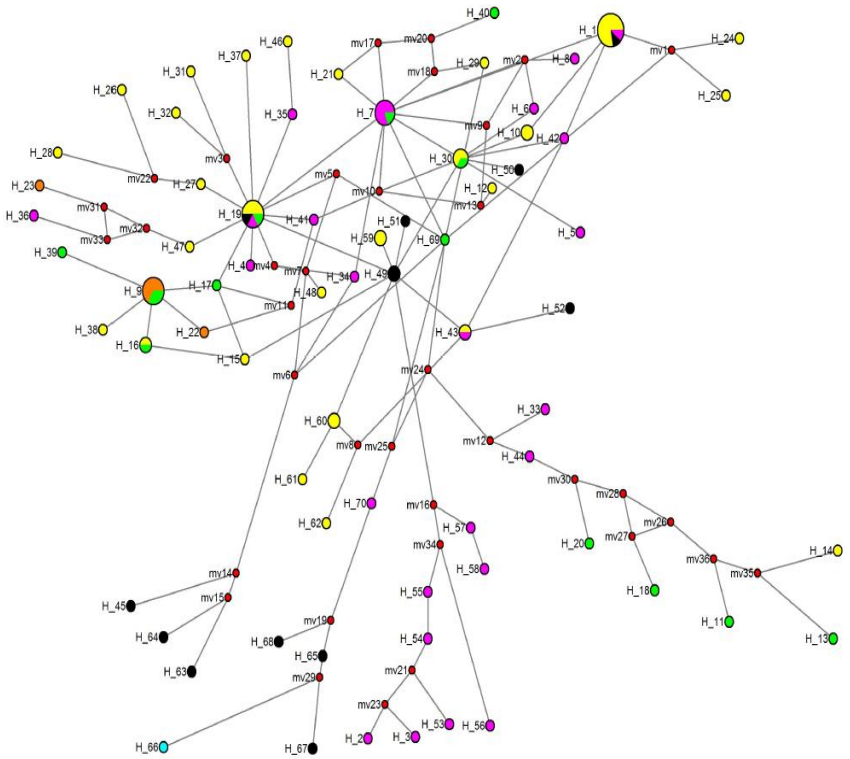

Figure 4. Median Joining Network (MJN) for the haplotypes detected in 100 tayra (*Eira barbara*) sequenced at the mitochondrial *ND5* gene. In light blue, one haplotype of *E. b. inserta*; in pink, haplotypes of *E. b. barbara*; in green, haplotypes of *E. b. peruana*; in yellow, haplotypes of *E. b. madeirensis*; in black, haplotypes of *E. b. sinuensis*; and in brown, haplotypes of *E. b. poliocephala*. Therefore, the five putative geographical subspecies of South American tayras and one Central America subspecies were represented in this analysis. Little red circles are extinct or not found haplotypes.

The BI temporal split estimate showed an initial divergence of the ancestors of the Panamanian individual (*E. b. inserta*) and South-American specimens around 6.26 MYA (95%: 5.4-8.49 MYA; Miocene divergence). The ancestor of the clade from northern Colombia split around 3.7 MYA (1.55-6.37 MYA). The ancestor of the animals from northwestern Argentina diverged around 3.15 MYA (1.01-4.23 MYA). In contrast, the ancestors of animals of north-central Peru, trans-Andean Ecuador and one of the Ecuadorian Amazonas clusters diverged 2.88 MYA (1.23-4.2

MYA), 2.35 MYA (0.31-2.7 MYA), and 1.49 MYA (0.42-3.74 MYA), respectively.

The MJN analysis revealed a view very similar to the phylogenetic trees (Figure 4). The major fraction of haplotypes were distributed irrespective of the geographical distribution of the morphological subspecies. For instance, the most frequent haplotypes (H1, H30, H7, H49, H19 and H9) included individuals of different putative subspecies: H1 contained exemplars classified "a priori" as *madereinsis*, *sinuensis* and *barbara*; H30 and H7 were composed of *madereinsis* and *peruana* individuals; H49 enclosed individuals of *sinuensis*; H19 consisted of specimens of *sinuensis*, *peruana*, *madereinsis*, and *barbara*, whilst H9 included *poliocephala* and *peruana*. Therefore, some haplotypes were widely distributed, which agrees quite well with extensive gene flow of this species across all of South America. Many of these main haplotypes presented other small haplotypes in star-like form, which is highly related to possible population expansions across the entire South American range of the tayra. Nevertheless, the MJN, as the phylogenetic trees, detected some haplotype clusters to be well delimited geographically. For example, there were the cases of the Central American individual (H66), the Cesar-Antioquia cluster (H65, H67, and H68), the trans-Andean Ecuadorian cluster (H45, H63, and H64), and the central Peruvian cluster (H11, H13, H18, and H20). The MJN temporal splits were slightly less than that those obtained with BI, but relatively similar. The temporal splits among these haplotypes and the main groups can be seen in Table 3. Some of these time splits are interesting. The divergence between the Panamanian individual and H7 was estimated to occur around 4.02 $\pm$ 0.31 MYA. The temporal divergence between clusters from the areas of northwestern Argentina, Cesar-Antioquia area (northern Colombia), trans-Andean Ecuador, and north-central Peru in reference to H7 were 3.73 $\pm$ 0.65 MYA, 3.29 $\pm$ 0.57, 1.04 $\pm$ 0.31 MYA, and 2.89 $\pm$ 0.47 MYA, respectively.

Therefore, the phylogenetics tree and the MJN analyses showed that the ancestor of Central and South American tayras originated during the Miocene or Pliocene. Also, the ancestors of some geographical groups, at least four of certain relevance, originated during the Pliocene and first part

of the Pleistocene. However, during the Pleistocene (as we will show later), tayra experienced a strong population expansion and many haplotypes expanded their geographical distributions. They superimposed onto the geographical areas of these older and geographical groups that originally differentiated during the Pliocene.

Genetic Distances and Genetic Heterogeneity among Putative Morphological Subspecies of *Eira barbara*

The Kimura 2P genetic distances among all of the comparison pairs of the six putative morphological subspecies of tayra are shown in Table 4. The differentiation between the Central American subspecies (*E. b. inserta*) and the five South American subspecies was elevated (5.5% - 7.8%), which confirmed that the Central America taxon is, at least, a different subspecies. It is interesting to note that the less differentiated South American taxon with regard to the Central American one was *E. b. sinuensis* (5.5 %). It was the South American taxon closest geographically. The genetic distances with the other four South American taxa ranged from 7.1%-7.8%.

In contrast, the genetic distances among the five South American subspecies were very small. They ranged from 0.1% to 1.2%. The pairs of subspecies with the greatest genetic distances were *E. b. poliocephala-E. b. sinuensis* (1.2%) and *E. b. poliocephala-E. b. barbara* (0.9%).

The overall genetic heterogeneity for all five South American tayra subspecies taken together was significant (Table 5), but the genetic heterogeneity was relatively small. For example, the F_{ST} and the γ_{ST} statistics showed values of 0.095 and 0.109, respectively. Their respective gene flow estimates of 4.75 and 4.10, were relatively high among the putative South-American subspecies.

Table 3. Temporal splits among different *Eira barbara*'s lineages estimated by means of a Median Joining Network (MJN). Values of temporal splits are in millions of years. SD = Standard deviation

Lineages compared	ρ ± SD	Temporal divergence
Between the Panamanian haplotype (*inserta*) and H49 (*sinuensis*)	13.000 ± 1.000	4.021 ± 0.309
Between the Bolivian and northern-western Argentinian haplotypes and H7 (*madereinsis, peruana*)	12.077 ± 2.097	3.735 ± 0.648
Between the Cesar-Antioquia (northern Colombia) haplotypes and H7 (*madereinsis, peruana*)	10.625 ± 1.829	3.286 ± 0.565
Between the trans-Andean Ecuadorian haplotypes and H7 (*madereinsis, peruana*)	3.375 ± 1.008	1.043 ± 0.311
Between the northcentral Peru and H7 (*madereinsis, peruana*)	9.333 ± 1.523	2.886 ± 0.471
Between H9 (*poliocephala, peruana*) and H19 (*sinuensis, peruana, madeirensis, barbara*)	1.000 ± 0.500	0.309 ± 0.154
Between H9 (*poliocephala, peruana*) and H7 (*madereinsis, peruana*)	1.363 ± 0.454	0.422 ± 0.140
Between H19 (*sinuensis, peruana, madeirensis, barbara*) and H7 (*madereinsis, peruana*)	0.454 ± 0.454	0.141 ± 0.141
Between H49 (*simuensis*) and H7 (*madereinsis, peruana*)	1.429 ± 0.714	0.441 ± 0.220
Between H30 (*sinuensis*) and H7 (*madereinsis, peruana*)	0.625 ± 0.625	0.193 ± 0.193
Between H9 (*poliocephala, peruana*) and H1 (*madeirensis, sinuensis*)	2.400 ± 0.600	0.742 ± 0.185
Between H19 (*sinuensis, peruana, madeirensis, barbara*) and H1 (*madeirensis, sinuensis*)	1.200 ± 0.600	0.371 ± 0.185
Between H49 (*sinuensis*) and H1 (*madeirensis, sinuensis*)	2.454 ± 0.818	0.759 ± 0.253
Between H7 (*madereinsis, peruana*) and H1 (*madeirensis, sinuensis*)	0.643 ± 0.643	0.198 ± 0.198
Between H30 (*sinuensis*) and H1 (*madeirensis, sinuensis*)	1.500 ± 0.790	0.463 ± 0.231

Table 4. Kimura 2P genetic distance (Kimura, 1980) in percentages (%) among six different morphological subspecies of *Eira barbara* (Mustelidae) (below main diagonal) and standard deviations in percentages (%) (above main diagonal) at the mt *ND5* gene. 1 = *E. b. barbara*; 2 = *E. b. peruana*; 3 = *E. b. madeirensis*; 4 = *E. b. poliocephala*; 5 = *E. b. sinuensis*; 6 = *E. b. inserta*; 7 = *Potos flavus* (Procyonidae)

	1	2	3	4	5	6	7
1		0.1	0.1	0.4	0.1	1.4	3.5
2	0.2		0.1	0.2	0.1	1.5	3.5
3	0.2	0.1		0.4	0.1	1.5	3.7
4	0.9	0.5	0.8		0.5	1.5	3.2
5	0.3	0.5	0.4	1.2		1.2	3.5
6	7.1	7.7	7.4	7.8	5.5		3.5
7	27.5	27.5	28.5	24.6	26.5	24.9	

Table 5. Overall genetic heterogeneity and gene flow (Nm) statistics for the five putative South American subspecies of *Eira barbara* at the mt *ND5* gene. * P < 0.05; ** P < 0.01

Estimated Genetic Differentiation	P	Gene flow	
$\chi^2 = 313.351$ df = 272	0.0429*	$G_{ST} = 0.0336$	Nm = 14.40
$H_{ST} = 0.0236$	0.0001**	$\gamma_{ST} = 0.0951$	Nm = 4.75
$K_{ST} = 0.0559$	0.0001**	$N_{ST} = 0.1108$	Nm = 4.01
$K_{ST}^* = 0.0362$	0.0001**	$F_{ST} = 0.1086$	Nm = 4.10
$Z_S = 2279.890$	0.0001**		
$Z_S^* = 7.360$	0.0001**		
$S_{nn} = 0.511$	0.0001**		

The analysis of subspecies pair comparisons with exact probability tests (Table 6) only showed two significant pairs: *E. b. madereinsis*-*E. b. barbara* (p = 0.0066 $\pm$ 0.0017) and *E. b. madereinsis*-*E. b. poliocephala* (p = 0.0135 $\pm$ 0.0034). In this analysis, the Central American taxon was deleted because only one sequence was analyzed. The estimates of Nm by

subspecies pair comparisons (Table 7) clearly yielded that the values lower than 1 (which is considered the limit for low gene flow; Wright, 1943) always implied the Central American taxon: *E. b. inserta-E. b. peruana* (Nm = 0.584), *E. b. inserta-E. b. barbara* (Nm = 0.459), *E. b. inserta-E. b. madereinsis* (Nm = 0.286), *E. b. inserta-E. b. poliocephala* (Nm = 0.137). Only a South American taxon, *E. b. sinuensis*, (Nm = 1.202) had a more substantial gene flow with *E. b. inserta*. This agrees quite well with that determined in the phylogenetic trees and in the genetic distance analysis. The gene flow estimates among the South American taxa were all above 1, ranging from 2.469 to 11.847. These values strongly correlate to elevated historical gene flows among the populations of tayra throughout South America.

Table 6. Exact probability tests (P) (below main diagonal) and standard deviations (above main diagonal) among six different putative morphological subspecies of *Eira barbara* by means of the mt *ND5* gene. 1 = *E. b. barbara*; 2 = *E. b. peruana*; 3 = *E. b. madeirensis*; 4 = *E. b. poliocephala*; 5 = *E. b. sinuensis*; 6 = *E. b. inserta*. * = Significant probability

	1	2	3	4	5	6
1		0.0000	0.0017	0.0138	0.0197	--------
2	1.0000		0.0071	0.0132	0.0088	---------
3	0.0066*	0.0679		0.0034	0.0201	---------
4	0.2307	0.3472	0.0135*		0.0036	---------
5	0.4044	0.4796	0.1032	0.0893		---------
6	---------	----------	----------	---------	----------	

Demographic Evolutionary Changes in the Tayra

All of the demographic change statistics indicated population expansion in the tayra (Strobeck's S statistic, P = 0.0001; Tajima D = -2.258, p = 0.0040; Fu & Li D* = -3.175, p = 0.0115; Fu & Li F* = -3.335, P = 0.0052; Fu's F_s = -52.632, P = 0.00001; and R_2 = 0.037, P = 0.0041).

Table 7. Gene flow (Nm) estimates (below main diagonal) among six different putative morphological subspecies of *Eira barbara* by means of the mt *ND5* gene. 1 = *E. b. barbara*; 2 = *E. b. peruana*; 3 = *E. b. madeirensis*; 4 = *E. b. poliocephala*; 5 = *E. b. sinuensis*; 6 = *E. b. inserta*

	1	2	3	4	5	6
1						
2	9.8245					
3	9.2893	11.8471				
4	2.6879	5.3236	2.2686			
5	7.1919	5.8729	4.8435	2.4691		
6	0.4597	0.5843	0.2862	0.1373	1.2019	

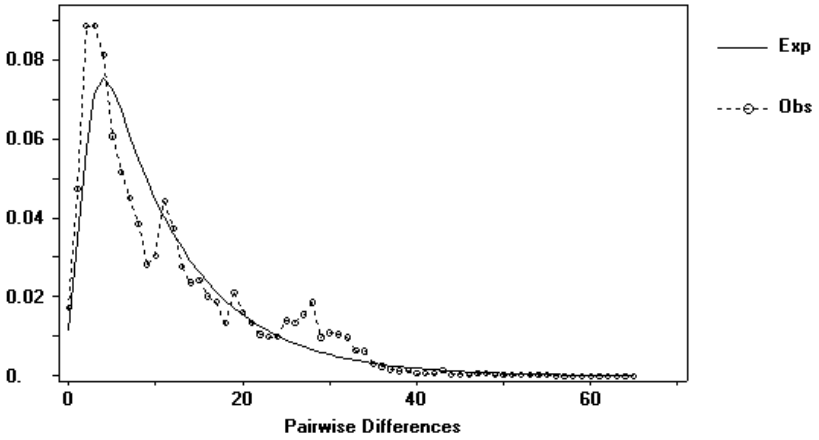

Figure 5. Historical demographic analysis by means of the mismatch distribution procedure (pairwise sequence differences) for the mitochondrial *ND5* gene studied in the overall sample of *Eira barbara*. The analysis showed a clear population expansion of this species during the Pleistocene.

The mismatch distributions also indicated population expansion (*rg* = 0.0040, P = 0.00280) (Figure 5). Assuming one year as one generation in the tayra, the population expansion began 343,586 YA, during the Pleistocene.

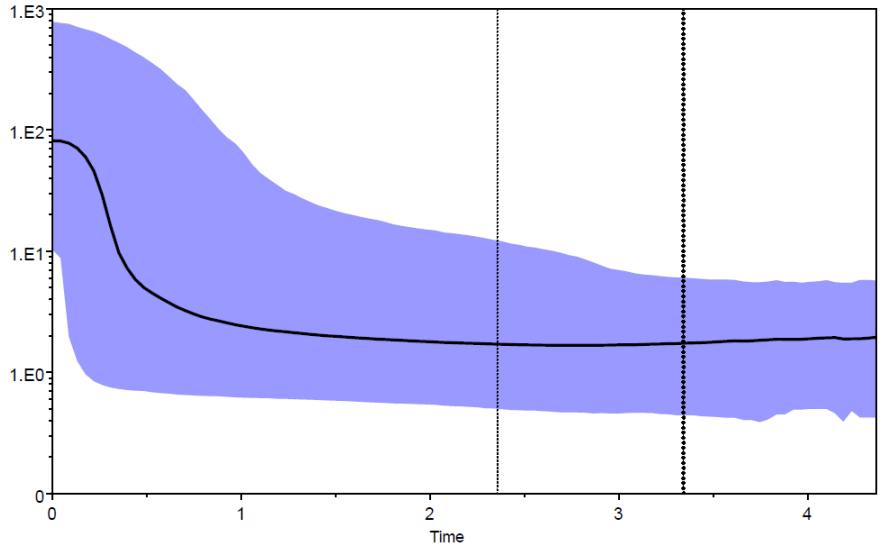

Figure 6. Bayesian skyline plot analysis (BSP) to determine possible demographic changes across the natural history of the overall sample of tayra (*E. barbara*) sequenced at the mitochondrial *ND5* gene. The analysis showed a clear female population expansion during the Pleistocene. On the x-axis, time in millions of years; on the y-axis, log effective population size of females.

The BSP analyses also determined a strong female population expansion during the Pleistocene for the tayra (Figure 6). The analyses showed the beginning of the expansion around 400,000 YA, very similar to the temporal estimate previously showed. Therefore, there is incontrovertible evidence that the tayra experienced a strong population expansion during the Pleistocene, as was previously suggested by the MJN analysis.

DISCUSSION

Genetic Diversity

The levels of nucleotide diversity found in *E. barbara* (π = 0.0422) were quite high. They were higher than those found in many other

neotropical carnivores. For example, they were higher than three fox species (*Lycalopex culpaeus*: π = 0.008, Ruiz-García et al., 2013a; *Lycalopex sechurae*: π = 0.015, Ruiz-García et al., 2013a; *Cerdocyon thous*: π = 0.019, Tchaika et al., 2006), three otter species (*Lontra felina*: π = 0.005, Vianna et al., 2010; *Lontra longicaudis*: π = 0.011, Ruiz-García et al., 2017a; *Pteronura brasiliensis*: π = 0.0086, Ruiz-García et al., 2017a), and two vulnerable Neotropical cats (*Leopardus jacobita*: π = 0.0047, Ruiz-García et al., 2013b; *Leopardus guigna*: π = 0.00461, Napolitano et al., 2013). The values of *E. barbara* were similar to those found in certain Neotropical cats, which are characterized by very elevated genetic diversity levels (*Puma yaguaroundi*: π = 0.0661; Ruiz-García and Pinedo-Castro, 2013 and Ruiz-García et al., 2017b; *Leopardus pajeros*: π = 0.0513, Ruiz-García et al., 2013b; *Leopardus pardalis*: π = 0.068, Eizirik et al., 1998; *Leopardus wiedii*: π = 0.035-0.074, Ruiz-García et al., 2017c).

These high levels of genetic diversity in "a priori" neutral markers, as that we studied, could be related with the fact that many other genes in the genome of a given species contains enough variability for the action of natural selection (Kimura, 1986). This could be the origin of the great morphological variation and behavior plasticity found in the tayra. Emmons and Freer (1990) determined that tayras could live in a wide variety of habitats such as tropical and subtropical forests, primary rain forest (as throughout the Amazonian forest in Brazil, Peru, Colombia and Ecuador), secondary rain and gallery forests (as in the Llanos of Venezuela and Colombia), gardens, plantations, and cloud forests. They also inhabit dry scrub and deciduous forests (as in the Pantanal in Brazil, Paraguay and Bolivia) and tall grass savannas (as in Argentina, Bolivia, and Paraguay). Sunquist et al., (1989) showed that the extreme plasticity of this species for habitat preferences, activity periods, and diet preferences may reduce interspecific competition between *E. barbara* and other carnivores. This could also be the explanation why Konecny (1989) found no significant habitat preference for this mustelid in Belize. The abundance of the tayra throughout much of Central and South America may be a consequence of its ecological flexibility compared to sympatric carnivores. Associated

with this, the tayra is a generalist predator, consuming a variety of fruits, carrion, small and medium vertebrates, insects, and honey (Cabrera and Yepes, 1960; Galef et al., 1976; Hall and Dalquest, 1963; Konecny, 1989; Sunquist et al., 1989).

Genetic Heterogeneity, Gene Flow and the Systematics of the Tayra

Our results clearly showed that the specimen sampled in Central America was highly divergent from all of the individuals sampled in South America. However, the genetic heterogeneity among the putative morphological subspecies of South American tayras, although significant, is very small as we found with the F_{ST} statistic, exact probability tests, and genetic distances. The indirect gene flow estimates were clearly higher than 1. Wright (1943) stated that in an island model, if Nm > 1, then gene flow is important enough to erase the genetic heterogeneity among populations. In a stepping-stone model, this amount must be larger than 4 (Trexler, 1988). In both models, Nm < 0.5 means that the populations are highly disconnected from a reproductive point of view. For instance, the gene flow estimates between *E. b. inserta* and *E. b. poliocephala* (Nm = 0.137), *E. b. inserta* and *E. b. madereinsis* (Nm = 0.286) and *E. b. inserta* and *E. b. peruana* (Nm = 0.459) showed that the Central American taxon is completely isolated from these three South American taxa. However, recall that certain genetic relatedness was detected between the Central American taxon and the most northern South American taxon (*E. b. sinuensis*). Additionally, we found several gene flow comparison pairs between South American taxa, such as *E. b. madereinsis-E. b. barbara* (Nm = 9.289) and *E. b. madereinsis-E. b. poliocephala* (Nm = 2.168). They were elevated, although these comparison pairs showed significant heterogeneity. This might be explained according to Alledorf and Phelps (1981), who argued that the most correct interpretation of Nm > 1 is that the populations share the same alleles, although not necessarily with the same allele frequencies. By means of simulations, these authors showed

that significant allele divergence occurred in 50% of the generations with a gene flow of Nm = 50. Significant allele divergence happened on most occasions when Nm = 10.

The tayra seems to have a strong dispersion capacity, which could be related with these high gene flow estimates detected for all the putative South American subspecies. For instance, in the Venezuelan and Colombian Llanos, tayras are usually found along gallery forests. However, tayras cross these extensive grasslands at night, presumably moving from one forest to another covering long distances (Defler, 1980).

Taking into consideration all these facts, we suggest that the six putative morphological subspecies analyzed could be reduced to two different subspecies: *E. b. inserta* for southern Central America and *E. b. barbara* for all South America. The name should be *E. b. barbara* because it was given by Linneus in 1758 versus *E. b. sinuensis* given by Humboldt in 1812, *E. b. peruana* given by Nehring in 1886, *E. b. madeirenis* given by Lonnberg in 1913 and *E. b. poliocephala* given by Traill in 1821. In reference to the putative northern Central America subspecies (*E. b. senex*), we cannot add any comment on its systematics because no individual of this putative subspecies was analyzed. Therefore, it is essential to sample tayras from this putative subspecies to determine its relationships with other tayra taxa.

Here we suggest another alternative point of view. As we showed, the first tayra's splits originated during the Miocene-Pliocene and beginning of the Pleistocene. However, during the Pleistocene, tayra experienced a strong population expansion and many haplotypes expanded their geographical distributions and they became superimposed on the geographical areas of older geographical groups that originally differentiated during the Pliocene. We suggest that future studies analyze nuclear genes to determine if there was hybridization between the older geographical groups (northern Colombia, part of Bolivia and northwestern Argentina, trans-Andean area of Ecuador, and north-central Peru) and the tayra's population that expanded throughout South America during the Pleistocene. If data support this, then our view of a unique tayra's subspecies in South America should be valid. On the contrary, if there was

little or no hybridization between the original groups and Pleistocene colonizers in sympatry, then the number of subspecies in South America could be higher. Therefore, the northern Colombian population (Cesar, Antioquia and possibly nearby areas) should be named *E. b. sinuensis* and the northern central Peruvian population should be named *E. b. peruana*. Also, the Bolivian and especially the northwestern Argentinian population should be defined as a new subspecies (tentatively *E. b. saltensis*). The trans-Andean Ecuadorian population should be defined as a new subspecies (tentatively *E. b. aequatorialis*). The remaining populations of tayra in South America should be named as *E. b. barbara*. Additionally, the range distribution of *E. b. sinuensis* and *E. b. peruana* should be more restricted than traditionally accepted (see Presley, 2000). Even, if some reproductive isolation mechanism had emerged between the Central and the South America tayras due to the old split estimated during the Miocene-Pliocene, both tayra populations should be consider two different species (*E. barbara* and *E. inserta*; this last should be *E. senex* if both Central American forms of tayra were genetically undifferentiated because *senex* was firstly named by Thomas in 1900 and *inserta* was named by Allen in 1908).

Only future nuclear genetic studies can clarify which of the two points of view is more acceptable.

Temporal Splits in the Tayras

Our results showed that the divergence between the Central and the South American tayras occurred around 6.3 to 4 MYA (Miocene or Pliocene periods depending of the temporal estimation). Johnson and O´Brien (1997) and Johnson et al., (2006) showed that seven of the eight primary lineages of felids radiated in the early part of the Late Miocene (10.8-6.2 MYA). There was a noteworthy cooling of the global climate near the end of the Middle Miocene. This period of cooling coincides with formation of a permanent Antarctic ice sheet in the Middle and in the Late Miocene and an Arctic ice sheet in the Pliocene. A large peak of

diversification in many vertebrate taxa occurred during the Pliocene epoch. The cold and dry climate during the Pliocene, coincides with the onset of high latitude glacial cycles, causing an explosive expansion of low-biomass vegetation, including grasslands and steppe at mid-latitudes and development of taiga at high latitudes of Eurasia and North America. These changes were correlated with the diversification of prey species such as muroid rodents and passerine birds that exploited these new habitats, which in turn provided new niches for little or medium carnivores, such as the tayra. Additionally, this Pliocene period agrees quite well with the last phase of rising of the Andes as shown by Dollfus (1974) and Clapperton (1993) (see, for instance, the rising of the "tablazos" of Piura, Peru) and very high volcanic activity in the Andes, with replacement of rainforests with steppe and grassland environments.

Our initial divergence estimates in the tayra agrees relatively well with other molecular studies in the reconstruction of the phylogenetic relationships in the Mustelidae (Bryant et al., 1993; Dragoo and Honeycutt, 1997; Koepfli and Wayne, 1998, 2003; Sato et al., 2003, 2004; Flynn et al., 2005; Fulton and Strobeck, 2006; Koepfli et al., 2008). Two molecular studies are fundamental to understanding the phylogenetics of the Mustelidae (Koepfli and Wayne, 2003; Koepfli et al., 2008). In the first study, the authors used five nuclear gene segments and the mt *Cyt-b* gene. The genes *APOB*, *FES*, *GHR*, *RH01* and *mtCyt-b* clustered *E. barbara* together with *Martes americana, Martes pennanti* and *Gulo gulo*. On the other hand, *CHRNA1* clustered *E. barbara* with *Meles meles* and *Arctonyx collaris*. The major part of the trees generated by these authors showed that Mustelinae and Melinae were polyphyletic within the Mustelidae, whereas Lutrinae was monophyletic. The authors of the second study analyzed 22 nuclear and mitochondrial gene segments and determined Mustelidae to consist of seven primary groups. These groups include four major clades and three monotypic lineages. It also included *Eira barbara* clustered into the subfamily Martinae, together with *Martes* and *Gulo*, the most divergent taxa within this subfamily (100 % of bootstrap and posterior probability of 1). In that study, the branch of *E. barbara* diverged from the other

Mustelidae around 6.7-7.7 MYA (calculated using the mean values), which agrees with our estimate.

These molecular results are not in conflict with the fossil record we know and understand for Mustelidae in America. Many species of Mustelidae appeared in North America during the Late Miocene-Early Pliocene. For instance, *Cernictis hesperus,* from the Pinole Tuff Local Fauna of California, has been dated radiometrically to have lived 5.3-5.5 MYA (Tedford et al., 2004; Baskin, 2011). Other cases of extinct genera appearances are *Trogonictis* and *Sminthosinis* as well as extant genera such as *Lutra* and *Mustela* during the Hemphilian period (4.7-5.9 MYA, Tedford et al., 2004). There is also *Legionarictis fortidens* from the Barstovian (Middle Miocene) marine Temblor Formation in California (Tseng et al., 2009). This form has shown a very close resemblance to other Miocene Mustelidae genera such as *Dehmictis, Eirictis, Iberictis,* and *Trochictis,* all from the Old World. The form also closely resembles *Sminthosinis, Trigonictis* (all from the New World), and, especially, with two extant genera, *Galictis* and *Eira* (Ray et al., 1981; Ginsburg and Morales, 1992; Baskin, 1998; Ginsburg, 1999). In fact, the cladistic analysis of Tseng et al., (2009) determined that this genus could be an evolutionary basal stage closely related to *Eira*. At the Longdan Fauna of the Gansu Province in China, a similar fossil to *Eira* was found by Qiu et al., (2004) from the Late Pliocene (*Eirictis robusta*; 2.58-2.15 MYA). However, the cladistic analysis of Tseng et al., (2009) determined that this mustelid was not very close to *Eira* and it is probably younger than *Eira*. Two possible fossil species of *Eira* have been described from post-Pliocene deposits of Maryland and Virginia under the names *Galera macrodon* and *G. perdicida*. However, the former has been assigned to *Trigonictis* based on additional material collected from deposits of the Blancan land mammal age from Washington, Idaho, Nebraska, Kansas, Texas, North Carolina, and Florida (Ray et al., 1981). *Trigonictis* is considered an intermediate form between *Galictis* and *Eira* and could be ancestral to both. The second species may be *Mephitis* (Alston, 1882). In addition, extinct species of *Eira* were noted from the Pliocene of the Eastern Hemisphere, but specific names were not given (Scott, 1937).

Hershkovitz, (1972) claimed that *Eira* and other endemic monotypic mustelid genera, such as *Lyncodon* and *Pteronura*, may have evolved in South America and moved north as part of the north and south American interchange across the Panamanian land bridge. In contrast, fossil records suggest that *Eira* may have a North American origin (Ray et al., 1981).

Therefore, the process of diversification within *Eira* could be at the end of the first mustelid diversification peak or at the beginning of the second mustelid diversification peak detected by Koepfli et al., (2008). In either case, this mitochondrial diversification process occurred before the Panamanian land bridge (3-2.5 MYA). Therefore, *Eira*'s could have radiated in North America before South America in concordance with the fossil record (Ray et al., 1981) and against the view of Hershkovitz (1972). If so, tayras arrived in South America before the complete formation of the Panamanian land bridge, coinciding with the Choco-Panama island bridge (Galvis, 1980), which could have been used by the ancestors of the current *E. barbara* to colonize northern South America from Central America. During the upper Pliocene orogeny, the present Tuira, Atrato and Sinu river basins and the nearby lowlands were raised above sea level. Thus, the mountains of southern Central America and of the northern Andes were uplifted to about their present elevation (Van der Hammen, 1961). Although the Nicaraguan, Panamanian and Colombian portals remained open (upper Miocene-middle Pliocene), numerous volcanic islands existed from the lower Atrato Valley and the Tuira River Basin of eastern Panama to the Nicaraguan portal. They could have been used by *Eira*'s ancestor to migrate southward. The Cuchillo Bridge of the Uraba region, connecting the Tertiary Western Colombian Andes with the Panamanian islands was probably above sea level during this period. Henceforth, tayras could be another "island hopper" species (Simpson 1950, 1965, 1980).

Our results could also be considered as indirect evidence of a Miocene origin of the Isthmus of Panama (Montes et al., 2012, 2015). Indeed, the Isthmus of Panama formation began earlier and seems to be associated with the Northern Andean uplift, around 24 MYA (Farris et al., 2011).

Therefore, the tayra could have arrived in South America before other Mustelidae. Koepfli et al., (2008) claimed that genera and species of

mustelids found in South America today are largely descended from North American immigrants that arrived as part of the GABI following the rise of the Panamanian isthmus, 3.0-2.5 MYA. Several informational points support the statement of Koepfli et al., (2008). 1-For example, there is the clade of New World otters where *Lutra canadensis* is sister to *Lontra felina*, and *Lontra longicaudis*. The latter two species are found in South America and are estimated to have diverged 2.8–3.4 MYA (95% HPD: 1.6–5.2 MYA) overlapping with the formation of the Panamanian land bridge. 2-The long-tailed weasel, *Mustela frenata*, ranges from North America to northern South America. In addition, *Mustela africana* and *Mustela felipei* are endemic to South America. Fossil evidence clearly indicates that *Mustela* colonized South America from the north, apparently well after the Panamanian isthmus was in place. 3- Fossils of the current *Lyncodon patagonicus*, (and a fossil form very related as *Lycodon bosei*) and *Stipanicicia* sp (closely related to the extant *Galictis cuja*) have been registered in the Ensenadean and Bonaerense periods of the Argentinian Pleistocene (Forasiepi et al., 2007). However our results could be ratified by other results provided by the same authors (Koepfli et al., 2008). They found that *Pteronura*, *Galictis* and *Eira* could have a Eurasian origin for each genus with posterior diversification in North America. For example, *Pteronura* may be related to the extinct genus *Satherium* from the Pliocene of North America. Additionally, *Eira* may be related to *Trigonictis* and *Legionarictis*, also North American fossils (Tseng et al., 2009). Fossil evidence suggests mustelids colonized the New World across Beringia during different intervals when the land bridge between Eurasia and North America was open. Multiple genera of mustelids migrated into North America during the Late Miocene (around 11.2-5.3 MYA), prior to the first opening of the Bering Strait 5.4–5.5 MYA, which severed the route across Beringia. Many genera that colonized North America during the Late Miocene or earliest Pliocene became extinct. However, *Eira* could be one of the surviving genera that began to diversify in North America and also in South America if we accept that they arrived in South America before the complete formation of the Panamanian land bridge.

The mitochondrial diversification of the oldest groups of South American tayra occurred 3.7-2.3 MYA. This coincided with the climatic changes that originated from the completion of the Panamanian land bridge (3.1–2.8 MYA; Marshall et al., 1979, 1982; Marshall, 1985, 1988; Webb, 1985, 1997; Coates and Obando, 1996) in the Last Pliocene. Diversification in the tayra occurred close to the Gelasian period (2.5–1.8 MYA), a period characterized by the last stages of a global cooling trend that led to the quaternary ice ages (International Commission on Stratigraphy 2007). Around 2.5 MYA, the Andean forests were transformed into open cold dry savannah ('paramo'), which could have potentially isolated populations of different species. They could have crossed the Northern Andes coming from Central America. Van der Hammen (1992) demonstrated that the mean temperature in the Colombian Andes was 4 ° C lower than today. He also stated that the rain level descended below the level reported for today (500–1,000 mm). At 2,500 meters above sea level (masl), the temperature was 10 ℃ lower than it is today. Tayra's diversification could have been affected by the rapid uplift that resulted in a significant elevation of the Northern Andes. The mountain range's height climaxed around 2.7 MYA when the northern Colombian Andes reached its present day elevation (Gregory-Wodzicki, 2000). This also coincides with the last formation of the Central Andes. All of the Andean chain between Cajamarca and Huancavelica in Peru appeared by volcanism in this period.

Much of the mitochondrial diversification process of the typical Pleistocene colonizer haplotypes occurred around 1.5-0.8 MYA. This divergence could have been initiated by the pre-Pastonian glacial period (1.3-0.8 MYA), which had the highest glacial peak of the first Quaternary glacial period (Günz). This glacial period was extremely dry, and there was a great degree of forest fragmentation. This period was a time for haplotype diversification. It was also a time of separation for many carnivores as it was previously determined for the Pampas cat (Cossíos et al. 2009), and for the foxes of the *Lycalopex* genus (Ruiz-García et al. 2013a). Around 1.3 MYA, the Buenos Aires's fauna transformed into a typical semi-arid Patagonian fauna, represented by the guanaco,

Lestodelphys and *Lyncodon*. Therefore, the climate was considerably colder and drier than today and could have influenced the mitochondrial fragmentation within the tayra.

The strong population expansion detected around 0.4 MYA for the tayra agrees well with an interglacial period (0.39-0.20 MYA, West 1967) characterized by higher temperatures and humidity and forest expansions (Hoxniense in the British Islands, Yarmouth in North America, Holstein in northern Europe and Mindel-Riss interglacial period in central Europe).

Future analyses with nuclear markers are needed as well as samples from Central America (especially, southern Mexico, Guatemala, Belize and Honduras), the Pacific areas of Colombia and Ecuador, other areas of Central Peru, and the Guyana shield. These markers and additional samples will help us to determine the exact number of subspecies or ESUs (Moritz, 1994). This information is crucial for the development of effective conservation plans for this species.

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